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oligonucleotides into eukaryotic cells

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Cationic Lipid Transfection

Transfection Methods Electroporation

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What is cationic lipid transfection?

Cationic lipid-mediated transfection is a fast, simple, and reproducible means for introducing DNA, RNA, siRNA, or

Cationic Lipid Transfection

Viral Transfection DEAE-Dextran Transfection

Calcium Phosphate Precipitation

Cationic Polymer Transfection

Other Physical Methods of Transfection

complex is taken up by the cells. This chemical transfection method allows the highly efficient delivery of nucleic acids into a broad range of cell types, including adherent, suspension, and insect cells, as well as primary cultures.
How lipid-mediated transfection works

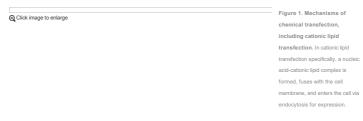
In this method, cationic lipids are mixed with nucleic acids in solution and added to cells, Next, the nucleic acid-lipid

Specially designed cationic lipids, such as Invitrogen Lipofectamine transfection reagents, facilitate DNA and RNA delivery into cells [1–3]. The basic structure of cationic lipids consists of a positively charged head group and one or two hydrocarbon chains.

Cationic lipid-based reagents spontaneously form condensed nucleic acid-cationic lipid complexes via electrostatic interactions between the negatively charged nucleic acid and the positively charged head group of the synthetic lipid.

In addition, some cationic lipid reagents are formulated with a neutral co-lipid or helper lipid, followed by extrusion or microfluidization, resulting in a unilamellar liposomal structure with a positive surface charge when in water. The positive surface charge of the liposomes mediates the interaction of the nucleic acid and the cell membrane, allowing for fusion of the liposome/nucleic acid transfection complex with the negatively charged cell membrane. The transfection complex is thought to enter the cell through endocytosis.

Once inside the cell, the complex must escape the endosomal pathway and diffuse through the cytoplasm. Transfected DNA is translocated to the nucleus to be expressed, while RNA or antisense oligonucleotides skip the translocation step and remain in the cytoplasm. Cationic lipids are thought to facilitate DNA transfection during the early steps of the process by mediating DNA condensation and DNA/cellular interactions.



Basic steps for performing cationic lipid transfection

	Step 2	Step 3	Step 4
Perform dilutions	Combine to form complexes	Add complexes to cells	Assay cells

Advantages of lipid-based transfection over other chemical transfection methods

The advantages of cationic lipid-mediated transfection are the ability to transfect a broad range of cell lines with high efficiency, its applicability to high-throughput screens, and the ability to deliver DNA of all sizes, as well as RNA and proteins. In addition, this method can be applied to both stable and transient expression, and unlike other chemical transfection methods, it can be used for *in vivo* transfer of DNA and RNA to animals and humans.

Moreover, many cell lines normally resistant to transfection by other methods can be transfected successfully with cationic lipid reagents.

Problems with traditional methods

Methods like calcium phosphate co-precipitation, DEAEdextran, polybrene, and electroporation include problems such as:

In contrast, lipid-mediated transfection:

- Yields high and previously unattainable transfection
 - efficiencies

 Works in a wide variety of eukaryotic cells
 - Is simple to perform
 - · Ensures consistently reproducible results
- Low efficiency of DNA deliveryPoor reproducibility
- Cell toxicity
- Inconvenience
- Inconvenience

The main drawback of cationic lipid-mediated transfection is the dependence of transfection efficiency on the cell type and culture conditions, requiring the optimization of transfection conditions for each cell type and transfection reagent.

Considerations for cationic lipid-mediated delivery

The first step for successful transfections is to choose the best transfection reagent for your application. Further optimization may be necessary. The table below will help you address some of these important factors so that you can easily achieve superior transfection results.

Transfection techniques for successful results

Considerations	Notes
Transfection in the presence of serum	 Serum, once thought to decrease transfection efficiency, can be present during transfection as long as the DNA-cationic lipid reagent complexes are formed in the absence of serum. Some serum proteins interfere with complex formation. The optimal amounts of cationic lipid reagent and DNA may change in the presence of serum; thus optimize conditions with serum if you plan to add it to the transfection medium. Most cells remain healthy for several hours in a serum-free medium.

Antibiotics in the culture medium	 Cationic lipid reagents increase cell permeability. This increases the amount of antibiotics delivered into the cells and results in cytotoxicity. Lower transfection efficiency may result. Therefore do not use antibiotics in the transfection medium. Avoid using antibiotics when plating cells for transfection. This reduces the need for rinsing the cells before transfection. For stable transfections, do not use penicillin and streptomycin in selective medium because the antibiotics are competitive inhibitors of Geneticin selective antibiotic. When creating stable cell lines, allow at least 72 hr for cells to express the resistance gene before adding selective antibiotic. If using serum-free medium, use lower amounts of antibiotics than you would in serum-containing medium to maintain the health of the cells.
Cell maintenance and evolution of cultures	 For optimal transfection results, follow a routine sub-culturing procedure. Passage cultures once or twice a week at a dilution that allows them to become nearly confluent before the next passage. Do not allow the cells to remain confluent for more than 24 hr. Cell cultures evolve over months and years in the laboratory, resulting in changes in cell behavior with regard to transfection. Thaving a fresh ampoule of cells allows recovery of transfection activity.
Cell plating density	 The optimal cell density for transfection varies for different cell types or applications. For adherent cells, generally 70% to 90% confluency at the time of transfection or 5 × 10⁵ to 2 × 10⁶ suspension cells/mL provides good results. Make sure cells are not confluent or in stationary phase at the time of transfection. Since transfection efficiency is sensitive to culture confluence, maintain a standard seeding protocol from experiment to experiment. In some cases, an increase in the number of cells plated increases the transfection activity.
DNA quality	 High-quality, intact plasmid DNA is important for achieving high-performance transfections. Thermo Fisher Scientific offers a line of nucleic acid purification kits. Cesium chloride banding yields highly purified DNA but is labor intensive and time consuming.

Cationic lipid-based transfection reagents

Thermo Fisher Scientific offers a wide selection of cationic lipid-mediated transfection reagents for efficiently introducing DNA, RNA, siRNA, or oligonucleotides into a broad range of cell types, including the Lipofectamine 3000 reagent.

Importantly, when selecting a transfection reagent, consider the payload you wish to deliver and the type of cells you want to transfect, because the choice of the transfection reagent strongly influences transfection results.

For more information on selecting the appropriate transfection reagent for your application, explore our transfection reagent selection auide.

TransfectionSelect tool

Find products, citations, and protocols optimized for your transfection experiments. Input information on your experiment type, cell line, and payload to unlock solutions.

Visit Transfection Basics to learn more about performing transfection in your lab.

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